R-Biopharm
RIDASCREEN FAST DON SC

Section	Page Number
GENERAL INFORMATION ……..…….....	1
PREPARATION OF TESTING MATERIALS……..…....	2
SAMPLE PREPARATION AND EXTRACTION PROCEDURES……..…….**.....**	2
TEST PROCEDURES……..…….	3
SUPPLEMENTAL ANALYSIS…….……..**.**	5
REPORTING AND CERTIFYING TEST RESULTS……..	5
STORAGE CONDITIONS AND PRECAUTIONS……..	6
EQUIPMENT AND SUPPLIES**...**…….	6
REVISION HISTORY……..…….	7

GENERAL INFORMATION

RIDASCREEN FAST DON SC is a competitive enzyme immunoassay for the quantitative analysis of deoxynivalenol (DON) in barley, corn, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn/soy blend, dried distillers grains, dried distillers grains with solubles, malted barley, milled rice, oats, popcorn, rough rice, rye, sorghum, soybeans, and wheat. All reagents required for the enzyme immunoassay, including standard, are contained in the test kit. The test is sufficient for 48 determinations (including standard). A microtiter plate spectrophotometer is required for quantification.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMB by phone at 816-891-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	*R-Biopharm Inc. 877-789-3033*
Test Kit Name:	RIDASCREEN FAST DON SC
Product Number:	R5905
Effective Date of Instructions:	11/13/2014
Instructions Revision Number	1
Conformance Range:	0.5 – 5.0 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	barley, corn, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn/soy blend, distillers dried grains, distillers dried grains with solubles, malted barley, milled rice, oats, popcorn, rough rice, rye, sorghum, soybeans, and wheat
Extraction method:	Shake vigorously 50 gram sample with 250 milliliters (mL) of deionized or distilled water for 3 minutes.
Test Format:	Microtiter well plate assay
Detection Method:	Stat Fax Reader, Model 303 Plus

PREPARATION OF TESTING MATERIALS

Wash Solution:

(1) To prepare the Wash Solution, dissolve the contents of the packet containing the buffer salt in 1 liter of distilled or deionized water. Document the technician's name, preparation date, and expiration date on wash solution bottle. A removable gum-label affixed to bottle for documentation is recommended.

(2) Swirl to mix before use. When stored properly (at 36 - 46º F) the solution has a shelf life of four weeks.

(3) Alternative Preparation of Wash Solution:

(a) Dissolve the contents of the packet in only 100 mL of distilled or deionized water to obtain a 10 fold concentrated washing buffer. This solution expires after approximately 8 weeks when store at room temperature (68 - 77º F).

(b) Use 1 part of the concentrated washing buffer and dissolve with 9 parts of distilled or deionized water to obtain the ready to use wash solution.

Example: 100 mL of this concentrated washing buffer should be mixed with 900 mL of distilled water to get 1000 mL of wash solution.

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

Standard Extraction Procedure for barley, corn, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn/soy blend, dried distillers grains, dried distillers grains with solubles, malted barley, milled rice, oats, popcorn, rough rice, rye, sorghum, soybeans, and wheat:

(1) Weigh 50 ± 0.2 grams ground sample into a whirl pack bag.

(2) Add 250 mL of distilled or deionized water and close the bag securely to prevent spillage.

(3) Shake vigorously by hand for three minutes.

(4) Let the extract sit for 2-3 minutes to allow for settling of the sample slurry.

(5) Filter the extract through Whatman #1 filters into a clean container that is labeled with a sample ID number.

(6) Dilute 1 part of the filtered extract with 3 parts of distilled/deionized water. (e.g., mix 1 mL filtered extract with 3 mL water). This is the **diluted filtrate extract** and ready for testing.

(7) Use 50 µL of the diluted filtrate extract per well for testing.

TEST PROCEDURES

a. Analysis Procedure

(1) Allow reagents and antibody wells to reach room temperature (68 - 77º F) prior to running the test.

(2) Only 1 control standard (zero standard) is included in the test kit. The standard curve (B/Bo) is provided with the certificate of the test kit.

(3) Insert a sufficient number of wells into the microwell holder for control standard and samples to be tested. (For example: to test 15 samples use 16 wells - 1 for the control standard and 15 for the test samples).

NOTE: Do not run more than 2 strips (15 samples) per run.

(4) Using a new pipette tip for the zero (0) control standard and each test sample, pipette 50µL of standard and prepared sample(s) to separate wells.

(5) Add 50 µL of enzyme conjugate (red capped bottle) into each well using a repeating pipettor with a 2.5 mL tip on setting 1.

(6) Add 50 µL of Anti-deoxynivalenol antibody (black capped bottle) into each well using a repeating pipettor with a 2.5 mL tip on setting 1.

(7) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.

(8) Incubate for **5 minutes** (± 1 minute) at room temperature.

(9) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(10) Using a wash bottle, fill each well with washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).

(11) Add 100 µL of substrate/chromogen (brown cap brown plastic bottle) to each well using a repeating pipettor with a 2.5mL tip on setting 2.

(12) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.

(13) Incubate for **3 minutes** (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(14) Add 100 µL of stop solution (yellow cap-brown glass bottle) to each well using a repeating pipettor with a 2.5 mL tip on setting 2.

(15) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.

(16) Measure absorbance at 450 nm using the Awareness Technology Stat-Fax Model 303 PLUS (results must be read within 10 minutes).

b. <u>Reading the Results</u>

(1) Stat-Fax Model 303 PLUS Microwell Reader.

(a) Press Menu, the prompt should read: "Select Test" press 1, then ENTER.

(b) The concentrations and B/BO% should now be printing.

(2) Display will read: "New B/BO Number Y/N (Yes/No). Press "N" if the B/BO matches the QC sheet in the test kit in use. Press "Y" if the B/BO on the printout does not match the QC sheet in the kit.

(3) If "Y" was pressed for new B/BO, it will now display: Cal 2 B/BO%=____simply insert the B/BO number from the QC sheet for standard 2 and press ENTER.

(4) When completed the reader will print "Test is Updated".

Note: Please verify new B/BO number entered on the printout match test kit QC sheet.

(5) If "N" was pressed for new B/BO, or you just finished updating the B/BO , it will now display: "Set carrier to 1; press Enter"

(6) Place the wells in the far right column of the carrier with the zero (0) standard being at the top.

(7) Align carrier to the far left for column 1. Then press ENTER.

(8) The reader is now reading the first eight wells. Once complete the display will read: "Plot Curve Y/N Select N.

(9) Display will now read: "Accept Curve Y/N.

If you are only running one strip, the test is now complete (press the clear button twice). If you have an additional strip to run, select yes. Move the carrier to the right so that the wells are aligned with notch in the center. Now press ENTER.

(10) The reader is now reading the second set of eight wells.

(11) Once the last strip is read, press the clear button twice.

(12) Test is now complete.

SUPPLEMENTAL ANALYSIS

Supplemental analysis is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative deoxynivalenol test kits is 0.5 – 5.0 ppm. Therefore, supplemental analysis would be performed for a result above 5.0 ppm. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 0.5 – 5.0 ppm for deoxynivalenol), and a correction for dilution is applied to derive at the final result. Supplemental analysis is performed only at the request of the applicant. A final result less than 3.5 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the sample extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 5.0 ppm.

Example: If the original analysis reported the DON value at 9.0 ppm and the conformance limit value is 5 ppm, dilute 1 mL of the diluted filtrate extract with 2 mL of distilled or deionized water (extraction solvent). The total volume is 3 mL. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the result obtained by 3 to obtain the final DON result. For example, if 3.1 ppm was the value obtained with the diluted extract, the final result in the original sample was 9.3 ppm (i.e., 3 x 3.1).

$$Final\ DON\ Result = Total\ Volume * DON\ Result$$

$$Total\ Volume = \frac{total\ dilution\ volume}{original\ volume}$$

Example:

$$Final\ DON\ Result = \left(\frac{3\ mL}{1\ mL}\right) *\ 3.1\ ppm$$

$$Final\ DON\ Result = 3 * 3.1 ppm = 9.3\ ppm$$

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-891-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

b. Precautions

(1) Do not interchange individual reagents between kits of different lot numbers.

(2) Do not use the test kits beyond the noted expiration date.

(3) The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits (48 well kit).

(1) 1 Microtiter plate with 48 wells (6 strips with removable wells each) coated with capture antibodies.

(2) 1 DON standard solutions of 1.3 mL 0 ppm (zero standard).

(3) 1 red-capped bottle of 3 mL peroxidase conjugated deoxynivalenol solution.

(4) 1 black-capped bottle of 3 mL anti- deoxynivalenol antibody.

(5) 1 brown-capped brown plastic bottle of 6mL substrate/chromogen, stained red.

(6) 1 yellow-capped brown glass bottle of 6mL stop solution.

(7) 1 packet of washing buffer (salt).

b. Materials Required but not Provided.

(1) Awareness Technology Inc. Stat-Fax Model 303 PLUS with 450-nm filter.

(2) RIDASOFT Win Software. (Optional)

(3) 50 µL, 100 µL, 1000 µL, and 5000 µl Pipettor and pipette tips.

(4) Graduated cylinders (plastic or glass): 0.25 and 1 liter.

(5) Sample shaker (optional).

(6) Filter funnel.

(7) Filter bags. (optional)

(8) Whatman #1 filter paper or equivalent.

(9) Balance.

(10) Repeating pipettor.

(11) Paper towels, Kay dry paper or equivalent absorbent material.

(12) Waste receptacle.

(13) Timer: 3 channel minimum.

(14) Waterproof marker, Sharpie or equivalent.

(15) Wash bottle.

(16) Deionized or distilled water.

REVISION HISTORY

Revision 1 (11/13/2014)

- Eighteen additional commodities (barley, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn/soy blend, dried distillers grains, dried distillers grains with solubles, malted barley, milled rice, oats, popcorn, rough rice, rye, sorghum, and soybeans) were approved for RIDASCREEN FAST DON SC test. The test procedure for these additional commodities has been incorporated in this revision.
- Removed Carl Jackson as contact.
- Added Ajit Ghosh as contact for the Technology and Science Division.